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Determination of Fatty Acid Composition of Two Carp Species by GC-MS

E. HORJ[1], A. IORDACHE[2], A. TOMA[3], O. COZAR[1] and M. CULEA[1,*]

[1]Babes-Bolyai University, 1 M. Kogalniceanu St., Cluj-Napoca 400084, Romania
[2]National Research and Development Institute of Cryogenics and Isotopic Technologies - ICSI Rm. Valcea, 4 Uzinei St., Rm. Valcea 240050, Romania
[3]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca-USAMV, 3-5 Manastur St., 400372 Cluj-Napoca, Romania

*Corresponding author: Tel: +40 264 405300; E-mail: monica.culea@phys.ubbcluj.ro

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A gas chromatography-mass spectrometric (GC/MS) technique was used to evaluate effects in quantitative fatty acids in fish plasma after Se addition in food, 0.03 mg/kg, in comparison with control. Selenium is a potent antioxidant protecting the body from damage due to oxidation by free radicals. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the major fatty acids found in fish. These fatty acids are produced by unicellular algae and phytoplankton which are consumed and then accumulate in fish. The aim of this work was to establish the extraction procedure and quantitative method of the fatty acids from fish plasma. The fatty acids were derivatized to obtain methyl esters. The identification of fatty acids was obtained by comparison of fatty acids methyl esters mass spectra with the mass spectra of fatty acids methyl esters kits and of NIST library. Concentrations of majority of unsaturated fatty acids increased with Se supplementation.

Key Words: Fatty acids, GC-MS, Derivatization, Selenium.

INTRODUCTION

The importance of the (n-3) poly unsaturated fatty acids (PUFA)[1] in human nutrition has led to considerable research effort in the last years. Subsequently, the efficacy of (n-3) PUFA in the prevention or attenuation of many of the inflammatory conditions that are prevalent in the developed world, including rheumatoid arthritis, atopic illness, inflammatory bowel disease, various neurological and cardiovascular diseases has been established. Fish and particularly those with oily flesh, such as herring, mackerel and salmon, represent a rich and almost unique source of the (n-3) PUFA, especially eicosapentaenoic acid [20:5(n-3), EPA] and docosahexaenoic acid [22:6(n-3), DHA]. Demand for fish products is increasing, yet the traditional capture fisheries are declining worldwide such that the potential shortfall in fish products must be met by aquaculture production. Future expansion of aquaculture and in particular salmon production, can continue only if suitable and sustainable alternatives to fish oil are developed and introduced[2].

A gas chromatography-mass spectrometry (GC/MS) technique was used for qualitative and quantitative analysis of fatty acids in fish. The method involves extraction procedure, derivatization and gas chromatography/mass spectrometric analysis (GC/MS). Fatty acids from plasma were derivatized as methyl ester derivatives[1-3]. Finally, the extracted analytes were detected by GC/MS in the electron impact (EI) mode[4].

The aim of the paper was to develop an analytical method for determination of fatty acids in biological samples. The method was validated by using fatty acid standard samples. The method was applied to evaluate effects in quantitative fatty acids in fish plasma after Se addition in food, 0.03 mg/kg, in comparison with control.

Theoretical: 20 µg of C11:1 internal standard was added after extraction and derivatization to each sample. The fatty acids were calculated according with the internal standard quantity and by using the response factors, for detector response correction. The response factors were obtained by repetitive injections into GC/MS of the standard mixture containing known quantity of each fatty acid. The fatty acids calculations in biological samples were performed following the formulae:

$$F = \frac{A_i / A_j}{m_i / m_j} \quad (1)$$

$$m_i(\mu g) = m_j(\mu g)\frac{A_i}{F_i \cdot A_j} \quad (2)$$

where m_i is the quantity corresponding to the compound i; m_j is the internal standard quantity added before sample preparation;

A_i and A_j are the peak areas of the compounds i and, respectively j; F_i are the response factors for compound i and j, respectively (the internal standard) calculated by using standards.

EXPERIMENTAL

General procedure: A trace DSQ Thermo Finnigan quadrupole mass spectrometer coupled with a Trace GC was used. The extracts were separated on a Rtx-5MS capillary column, 30 m × 0.25 mm, 0.25 µm film thickness, using a temperature program from 50 ºC, 1 min, 8 ºC/min at 300 ºC, the flow rate 1 mL/min, with helium 5.5 as carrier gas. Undecaenoic acid (C11:1) was used as internal standard. The following conditions were followed: transfer line temperature 250 ºC, injector temperature 250 ºC; ion source temperature 250 ºC; splitter: 10:1. Electron energy was 70 eV and the emission current, 100 µA.

The fatty acids were extracted from the plasma samples after amino acids extraction on a Dowex 50W-W8 exchange resin. 2 mL water were also passed and collected. The extraction was then performed by adding 0.5 mL chloroform:methanol (2:1) and mixing 30 s. Then was followed a centrifugation for 5 min. The supernatant was removed. The extract was dried with a nitrogen flow. Derivatization procedure was applied for esterification with methanol-acetyl chloride (4:1 v/v) for 20 min at 80 ºC. The method was validated. The validation parameters precision and sensitivity were tested. GC/MS analyses were performed for the determination of fatty acids in carp fish plasma. Two different varieties of carps (*Cyprinus carpio*) were studied: Galitian and Lausitz. The fodder consisted of: 38 % proteins, 5 % fat, 3.5 % pulp and 9 % humidity. For the experimental batch the fodder included also 0.03 mg organic Se (Sel-plex, All-Tech, USA) per kg. Plasma samples were collected from each carp, 8 controls and 10 experiments of the two varieties.

RESULTS AND DISCUSSION

The developed method is selective and specific. The mass spectra recorded on each chromatographic peak permit the precise identification of the fatty acids, by using NIST library of mass spectra. Also, the overlapping of the compounds is easily discovered. The method was validated by using fatty acid standards. Precision gave lower value than 10 % for RSD and sensitivity value was lower than 10 ng of fatty acid injected. All the samples followed the same extraction and derivatization steps.

Fig. 1 presents the separation chromatogram of fatty acids methyl esters (FAME) in a plasma fish sample. The comparison of the separated FAME from different carp fish extracts showed very similar profiles, as could be observed in Fig. 2. Plasma concentrations of fatty acids were measured and compared in carp fish plasma, after Se addition in food, 0.03 mg/kg, in comparison with control.

The fatty acids identified in the plasma samples of the carp fish are shown in Table-1. The analysis of the fatty acid composition revealed that polyunsaturated fatty acids (PUFA) were the most representative (*ca*. 53 %), followed by saturated fatty acids (SFA) (*ca*. 24 %) and finally by monounsaturated fatty acids (MUFA) (*ca*. 21 %). Among MUFA, monoenic 16:1

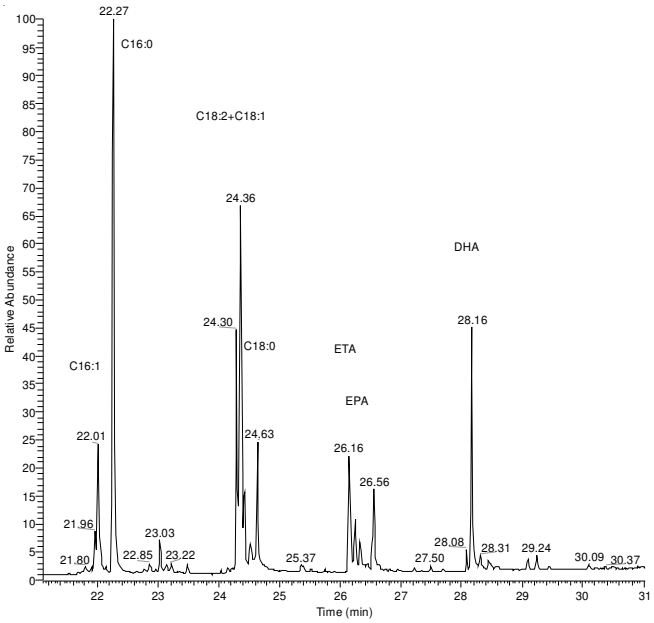


Fig. 1. Separation chromatogram of the important fatty acids methyl esters identified in fish plasma

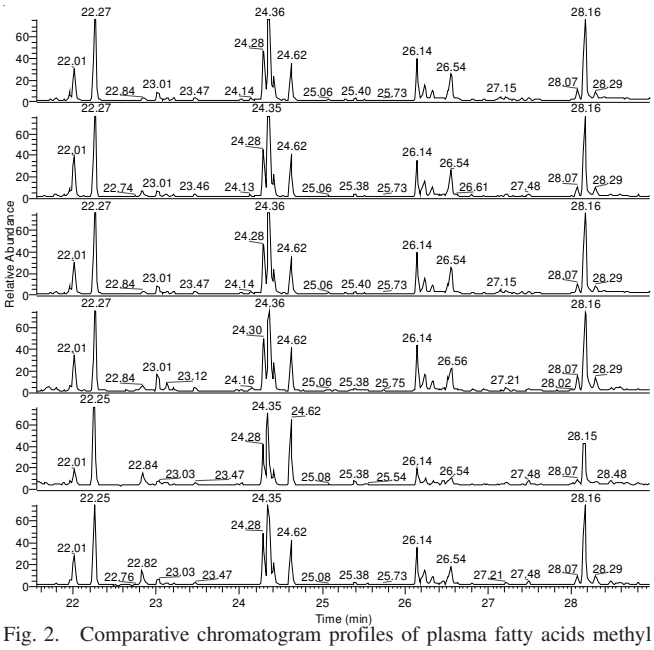


Fig. 2. Comparative chromatogram profiles of plasma fatty acids methyl esters from different carp fish extracts

(ranging from 5-8 %) and 18:1ω9 (ranging from 14-21 %) formed a considerable percentage of the total fatty acids. The dominant species of SFA were 16:0 (ranging from 15-30 %) and 18:0 (ranging from 6-9 %). EPA (20:5ω3) and DHA (22:6ω3) were the dominant PUFA and percentages were 30 % (Table-1). No significant differences were found for the majority of fatty acids in the two varieties of carp.

Figs. 3-5 presents the comparison fatty acids in experimental and control of the two varieties of carps. Significant increase of DHA and unsaturated fatty acids (UFA) in both varieties of experimental plasma was observed in comparison with control (Figs. 4 and 5).

Eicosapentaenoic acid and docosahexaenoic acid, found only in fish and other sea foods, possess profound beneficial properties for human health. In this research, significantly

TABLE-1
FATTY ACIDS COMPOSITION IN CARP FISH PLASMA. THE VALUES ARE EXPRESSED AS PER CENT OF TOTAL FATTY ACIDS (n = 10)

FAME	T_R	Mean ± DS
Hexadecenoic acid (C16:1)	22.01	6.84 ± 1.21
Hexadecanoic acid (C16:0)	22.27	18.61 ± 4.53
9,12-Octadecadienoic acid (C18:2)	24.28	8.05 ± 2.04
9-Octadecenoic acid (C18:1)	24.36	17.59 ± 2.20
Octadecenoic acid (C18:1)	24.41	3.85 ± 0.48
Octadecanoic acid (C18:0)	24.62	6.28 ± 0.62
5,8,11,14-Eicosatetraenoic acid (C20:4) ETA	26.14	7.68 ± 1.20
5,8,11,14,17-Eicosapentaenoic acid (C20:5) EPA	26.22	3.76 ± 1.03
ETA	28.07	2.85 ± 1.36
(C22:6)DHA*	28.16	14.85 ± 6.46
ETA	28.29	1.18 ± 0.58
Σ SFA	–	23.53
Σ MUFA	–	21.42
Σ PUFA	–	53.17
Σ USFA/ ΣSFA	–	3.2
DHA /EPA	–	4.3

*4,7,10,13,16,19-docosahexaenoic acid

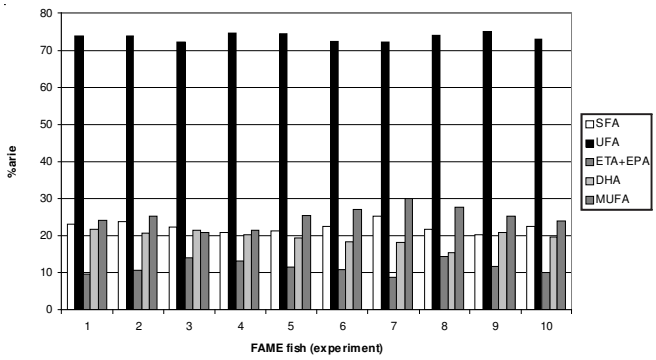
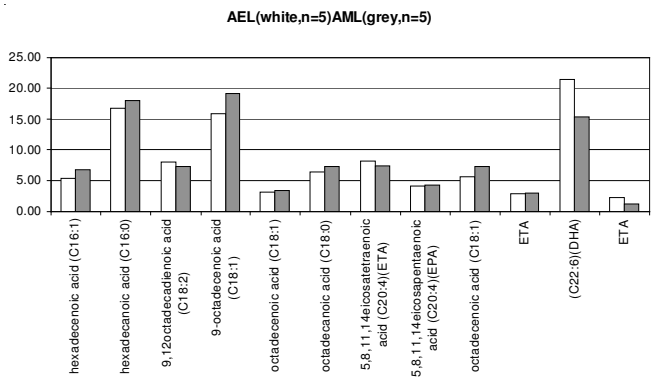


Fig. 3. Comparison among SFA, UFA, ETA +EPA, DHA and MUFA in experimental fish

($p < 0.01$) higher docosahexaenoic acid content was determined in the plasma samples of experimental carps studied *versus* control.

Conclusion

The method developed showed good precision in the analysis of fatty acids from plasma samples. The high values of poly unsaturated fatty acid in fish plasma prove the high nutritious value of fish. Docosahexaenoic acid increase in plasma samples of experimental fish could be related with the antioxidant properties of selenium in the body.



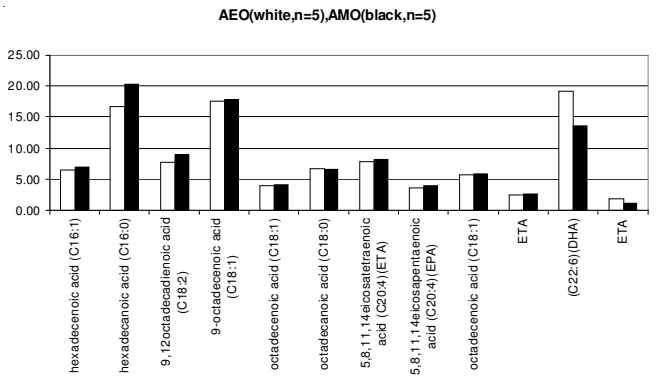


Fig. 4. Comparison of changes of fatty acids in experimental and control carp of two varieties (AEL: fatty acid levels in plasma of experimental Lausitz carp and AEO: fatty acid levels in plasma of experimental Galitian carp; AML and AMO: fatty acid levels in plasma of control Lausitz and Galitian carp)

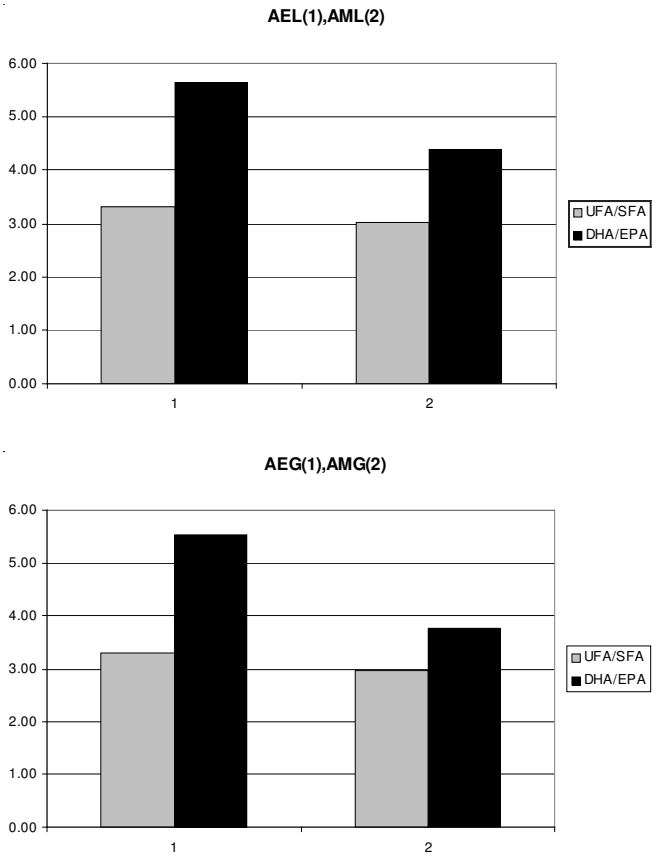


Fig. 5. Comparison of UFA/SFA and DHA/EPA ratios in experimental and control fish of two varieties

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